Detection of CYP2C11 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Block: Protein Block Serum-Free Ready-To-Use
Dakocytomation USA
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Catalog # X0909

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary antibody: Rabbit Polyclonal to Cytochrome P450 2C11 Antibody
Abcam Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab3571

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

LSAB+ System-HRP
Dakocytomation USA
Carpinteria CA 93013
www.dakousa.com
Catalog #K0690
* This kit contains all the reagents necessary for secondary and label antibodies.

Staining Procedure

Positive Control Tissue: Rat Liver (upregulated)
Stain Localization: Centilobular cytoplasmic staining pattern

Deparaffinize and hydrate slides through the following solutions:

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques using the microwave oven.
   Place a full rack of slides in tissue tekô container containing 200ml of distilled water.
   Microwave for 5 minutes at level 5
   Cool for 1 minute (Add more distilled water if necessary)
   Microwave for 5 minutes at level 5. Temp after Microwaving_____
   Cool 20 minutes at room temperature.
   Rinse in distilled water two times for 3 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Incubate slides in Dako Serum-Free Protein Block for 10 minutes at room temperature.
   Lot#_____ Exp. Date_____

   DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

6. Apply Avidin/Biotin block
   Lot#_____ Exp. Date_____New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X Automation Buffer

Apply biotin block - 15 minutes at room temperature.
Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cyp2C11) at a 1:100 dilution and incubate for one hour at room temperature.
   Lot#_____ Date Aliquoted_____

   For negative control slides, normalize the normal rabbit serum to the protein concentration of the primary antibody (Cyp2C11), and use this to make a 1:100 dilution. Apply to the slides and incubate for one hour at room temperature.
   Lot#_____ Reconstituted Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

   LSAB+ Kit Lot#_____ Exp. Date_____

9. Apply Link – Secondary (yellow bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label (red bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
    (Add 1 drop of DAB per ml of substrate)
    Lot#_____ Exp. Date_____ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 08/21/06